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# Optimization of sample preparation for MRI of formaldehyde-fixed brains

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**Target audience:** researchers who perform MR acquisitions on formaldehyde-fixed post-mortem brains.

**Purpose:** Magnetic resonance imaging of post-mortem brains allows long acquisition times up to several days and can be used to obtain high-resolution images at high field (7T) which can be readily correlated with histological examination of the tissue. However, death and formaldehyde fixation are known to modify severely the relaxivity and diffusion properties of brain tissue.<sup>1,2</sup> In particular, formaldehyde is known to shorten  $T_2$ , which drastically reduces SNR.

In order to counteract this effect and recover better SNR, free fixative can be washed out by soaking the sample in isotonic saline solution. This has been demonstrated in small biopsy-sized tissue samples,<sup>3</sup> but little data is available concerning whole brain specimens.<sup>2</sup>

This study was designed to describe the kinetics of the change of relaxivity and diffusion properties of whole brain specimen at 7T, during fixation, and during soaking in saline solution, in order to determine optimal soaking times.

**Methods:** Two healthy ewes (adult female sheep *ovis aries*, 2 years old) were used for this study, in accordance with local animal regulation (Authorization A37801 at the French Ministry of Agriculture). The animals were euthanized through massive injection of barbiturate, after having received heparin to prevent coagulation. The head was separated from the body, and perfused through the carotid arteries with 40L of PFA at 4°C (4% formaldehyde in phosphate-buffered saline, prepared from paraformaldehyde powder). The perfusion happened within a few minutes of death, hence no degradation should happen during the post-mortem interval (PMI) between death and fixation. The brain was then extracted, immersed into PFA for post-fixation, and kept at 4°C before the first scan.

Samples were scanned repeatedly (23 and 19 times respectively during post-fixation of both samples in PFA, 19 times during saline soaking for one sample) on a clinical 7T MRI system, mapping the following quantitative parameters over the whole brain.  $T_1$  was mapped using variable flip angle–actual flip angle imaging<sup>4</sup> (VAFI) at 10mm isotropic resolution.  $T_2$  was mapped at 1.7mm isotropic resolution using spin echo EPI repeated several times for a large window of TE.  $T_2^*$  was mapped using a multi-echo gradient echo sequence with 10mm isotropic resolution. Both  $T_2$  and  $T_2^*$  were fitted with least-squares regression of a single exponential decay. Diffusion-weighted images were acquired using single shot spin echo EPI with 20mm isotropic resolution, 256 diffusion directions,  $b = 4500\text{s/mm}^2$ . Mean diffusivity and fractional anisotropy were calculated with a first-order tensor model. Regions of interest were drawn manually in 3D in sub-cortical white matter and caudate nuclei (see Figure 1), and were transformed into the referential of each quantitative map using rigid registration. Voxels affected by partial volume or artefacts due to air bubbles were excluded.

**Results:** The time course of the measured parameters is represented on Figure 2, over 10 weeks during fixation and saline soaking. The data for caudate nuclei is noisy with a few outliers, because they contain very few voxels for lower resolution measurements.  $T_1$  decreases during fixation for approximately 8 weeks, and is restored only marginally by saline.  $T_2$  and  $T_2^*$  decrease for the first week of fixation, then grow back to a plateau in approximately 6 weeks. They grow during the first two weeks during saline soaking, then seem to decrease very slowly. The mean diffusivity decreases for the first week of fixation, and increases slightly in saline. Fractional anisotropy does not change significantly during the whole experiment.

**Discussion:** Good reproducibility is observed between subjects where the curves overlap. The slow decrease of  $T_1$  is consistent with previously published data,<sup>1</sup> as well as the decrease of diffusivity and stability of anisotropy.<sup>2</sup> On the other hand, the rebound of  $T_2$  and  $T_2^*$  after one week was not expected. A similar phenomenon was previously attributed<sup>5</sup> to early decomposition of unfixed deep tissue. However, the present study uses perfusion fixation, which should stop such degradation by exposing all the tissue to fixative. Instead, we hypothesize that this rebound is due to the degradation of the PFA solution itself through precipitation of monomeric formaldehyde into paraformaldehyde, effectively lowering the formaldehyde concentration as the solution degrades. This could be avoided by regularly changing the fixative or by using formalin, which includes methanol to prevent polymerization of formaldehyde. Washing the tissue in saline had the intended effect of restoring higher  $T_2$ ,  $T_2^*$ , and diffusivity, although to a rather modest degree, which can be explained if the fixative concentration decreased as hypothesized.

**Conclusion:** The usefulness of the saline soaking procedure is assessed in terms of restoring  $T_2$ ,  $T_2^*$ , and diffusivity, which is particularly beneficial to post-mortem diffusion imaging. The fixation was found to stabilize after approximately 8 weeks, and the optimal duration of saline soaking is found to be around 3 weeks. These durations can be expected to be longer for larger specimen, such as human brains, which require longer penetration times.

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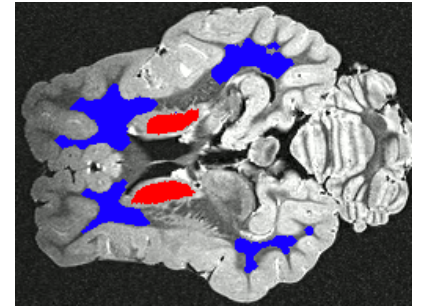


Figure 1: 2D snapshot of a high-resolution Turbo Spin Echo scan of one subject and associated 3D ROIs.

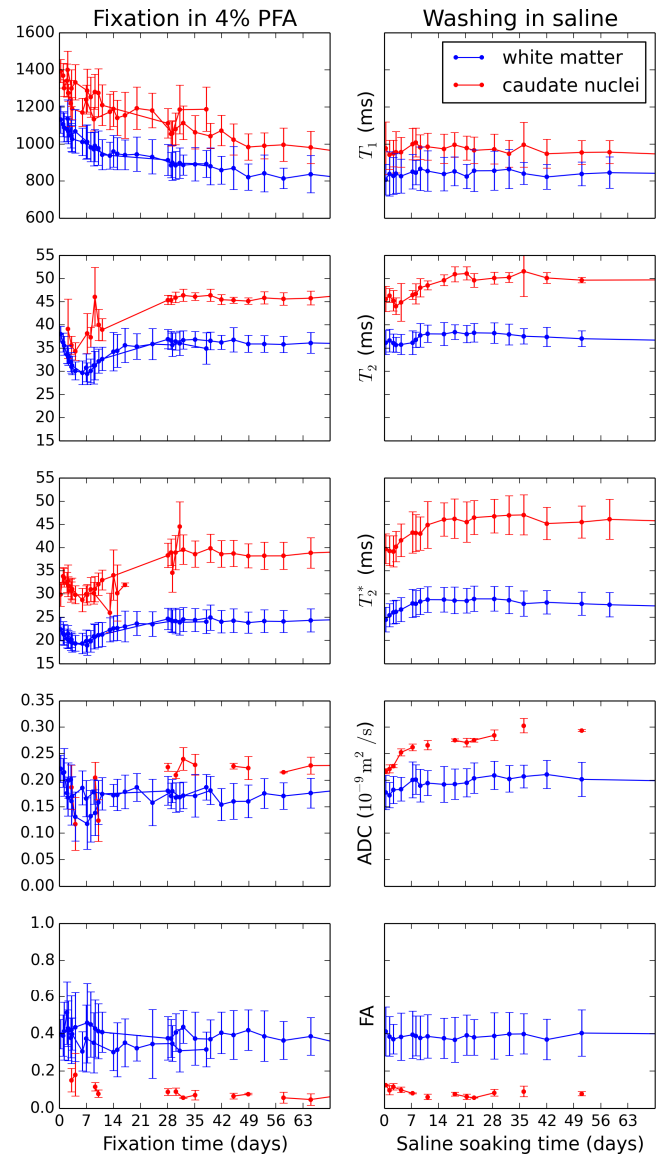


Figure 2: time course of measurements during fixation and saline soaking. Error bars represent mean  $\pm$  standard deviation in each ROI. Each subject is represented by one curve.